Parvalbumin interneuron ErbB4 controls ongoing network oscillations and olfactory behaviors in mice

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Abstract

Parvalbumin (PV)-positive interneurons modulate the processing of odor information. However, less is known about how PV interneurons dynamically remodel neural circuit responses in the olfactory bulb (OB) and its physiological significance. This study showed that a reinforced odor discrimination task up-regulated the activity of ErbB4 kinase in mouse OB. ErbB4 knock-out in the OB impaired dishabituation of odor responses and discrimination of complex odors, whereas odor memory or adaptation had no alteration in mice. RNAscope analysis demonstrated that ErbB4-positive neurons are localized throughout the OB, whereas within the internal and external plexiform layers, ErbB4 mRNA are largely expressed in PV-positive interneurons. ErbB4 knock-out in PV interneurons disrupted odor-evoked responses of mitral/tufted cells, and led to increased power in the ongoing local field potential in awake mice. We also found a decrease in the frequency of miniature inhibitory postsynaptic currents and deficits in stimulus-evoked recurrent and lateral inhibition onto mitral cells, suggesting broad impairments in inhibitory microcircuit following PV-ErbB4 loss. Similarly, ErbB4 ablation in OB PV interneurons disrupted olfactory discrimination and dishabituation in mice. These findings provide novel insights into the role of PV-ErbB4 signaling in inhibitory microcircuit plasticity, ongoing oscillations, and OB output, which underlies normal olfactory behaviors.

Introduction

Discriminating chemical cues in the environment is not only necessary for evaluating potential threats but also influences cognitive and emotional behaviors. The olfactory system dynamically adjusts its sensitivity to odors with changes in the environment and motivational and cognitive states. For example, odor learning improves performance on olfactory discrimination tests. Habituation and adaptation to successive exposures to an odor lead to decreased behavioral responses to it, whereas an unexpected odor enhances olfactory salience and stimulates behavioral dishabituation. Dysfunction in olfactory discrimination has been observed in the earliest stages of several neurodegenerative disorders and psychiatric diseases (Carnemolla et al., 2020; Walker et al., 2021). Similarly, olfactory discrimination ability has significant predictive valence for future cognitive decline in healthy aging individuals (Sohrabi et al., 2012; Uchida et al., 2020; Yoshitake et al., 2022). However, neural mechanisms that underlie the dynamic modulation of olfactory behaviors remain largely unknown.

The olfactory bulb (OB) is the first relay and processing station in the olfactory system. In the OB, odor signals are transformed into the informational output that is sent to the associated olfactory cortex. As the principal output neurons of the OB, mitral/tufted cells (M/TCs) receive glutamatergic excitatory inputs from olfactory sensory neurons and GABAergic inhibitory inputs from heterogeneous subpopulations of interneurons. An imbalance in excitation/inhibition onto MCs has been shown to impair olfactory discrimination (Lepousez and Lledo, 2013). Furthermore, overlapping odor-evoked input patterns to the OB undergo reformatting

to enhance discrimination of similar odors, and this reformatting depends on GABAergic interneurons (Gschwend et al., 2015; Li et al., 2018; Mohamed et al., 2019; Zavitz et al., 2020). GABAergic interneurons in the OB mediate recurrent and lateral inhibition of MCs. Recurrent inhibition of MCs by granule cells (GCs) increases the frequency of odor-induced activity and facilitates odor discrimination (MacLeod and Laurent, 1996; Nunes and Kuner, 2015). Interglomerular lateral inhibition sharpens or filters the odor-induced output signals of MCs, enhancing the contrast of odor representations and consequently the ability to discriminate similar odors (Egger and Kuner, 2021). Overall, the OB possesses various types of interneurons to control different olfactory behaviors (Lyons-Warren et al., 2023; Pardasani et al., 2023; Takahashi et al., 2016). Parvalbumin-positive (PV) interneurons form dendrodendritic contacts with the somata and dendrites of neighboring MCs (Huang et al., 2013; Kato et al., 2013; Matsuno et al., 2017; Miyamichi et al., 2013). Unlike odor-specific responses of GCs and MCs, PV interneurons exhibit strong responses to broader odors (Kato et al., 2013). M/TCs receives prominent input of PV interneurons from external plexiform layer (EPL) (Miyamichi et al., 2013). However, the contributions of PV interneurons to olfactory behaviors and the associated molecular mechanisms are not understood.

The receptor tyrosine kinase ErbB4 is preferentially expressed by GABAergic neurons in cortical areas, but in subcortical regions, ErbB4 is also expressed by non-GABAergic neurons (Bean et al., 2014; Deng et al., 2019; Dominguez et al., 2019; Fazzari et al., 2010; Lin et al., 2018; Mei and Nave, 2014; Shi and Bergson, 2020). ErbB4 kinases are expressed in neuroblasts of the subventricular zone and the rostral

86 migratory stream to the OB, implicating ErbB4 signaling in olfactory interneuronal
87 precursor differentiation and olfaction (Anton et al., 2004). ErbB4 regulates synaptic
88 transmission and plasticity in PV interneurons in the neocortex and hippocampus
89 (Chen et al., 2022; Dominguez et al., 2019; Grieco et al., 2020; Lin et al., 2018).
90 Recently, we found a distinct ErbB4 signaling pathway in PV interneurons of the
91 prefrontal cortex that hinders flexibility in odor-associated memory updating but has
92 no influence on the initial olfactory learning and memory (Cai and Shuman, 2022; Xu
93 et al., 2022). However, whether or not ErbB4 is expressed by PV neurons in the OB,
94 and its effects on olfactory behaviors if so, are not clear.

95 In this study, we show that an odor discrimination task promotes the activation of
96 ErbB4 in the OB. ErbB4 mRNA-positive neurons are localized throughout the OB
97 layers, with most being PV-positive interneurons in the internal plexiform layer (IPL)
98 and EPL. In this context, we evaluated the performance of PV-ErbB4 knockout mice
99 in odor discrimination, habituation/dishabituation, and detection threshold tasks. We
100 also explored the effects of ErbB4 in PV interneurons on the odor-evoked activity of
101 MCs and ongoing network oscillations in awake mice, and on the inhibitory
102 modulation onto MC output. Our findings reveal physiological roles and mechanisms
103 of PV interneuron ErbB4 signaling in dynamic modulation of olfactory behaviors.
104

105 **Results**

106 **Performing an odor discrimination task upregulates the activity of ErbB4 in the**
107 **OB**

First, we examined the levels of ErbB4 protein and its phosphorylated form (p-ErbB4) in mouse OB after a reinforced go/no-go odor discrimination task. Over 3 days (200 trials each day), adult mice were first trained to discriminate between a pair of simple odors (water-rewarded isoamyl acetate and unrewarded heptanone) and then learned to discriminate complex chemical cues (60%/40% binary mixtures of these odors) (Figures 1A and 1B). A similar learning task without odor discrimination (go/go task) was performed in the control group (Figure 1A). As shown in Figures 1C and 1D, the level of p-ErbB4 in the go/no-go group was higher than that in the control group, suggesting a link between increased ErbB4 activity in the OB and olfactory discrimination ability.

ErbB4 in the OB is critical for odor discrimination and dishabituation

Next, we evaluated the performance of ErbB4 knockout mice in the go/no-go task. ErbB4 protein was specifically deleted in the OB by delivery of adeno-associated virus recombinants expressing Cre recombinase and green fluorescence protein (GFP) (AAV-Cre-GFP) into both sides of the OB of neonatal mice carrying *loxP*-flanked *ErbB4* alleles (Figure 1E and F). When learning to discriminate simple odor pairs (Figure 1G), both AAV-GFP and AAV-Cre-GFP mice reached an accuracy of above 90% within 3 days (~ 600 trials), suggesting that ErbB4 deletion in the OB has no influence on learning and memory or the sense of smell. However, when distinguishing the 60/40% binary mixtures of these odors, the AAV-GFP group showed an accuracy above 80% after the 4-day training program (~ 800 trials), but the

AAV-Cre-GFP-injected mice showed a significant lower accuracy. This indicates that ErbB4 in the OB maintains the ability of mice to discriminate complex odors.

To further determine the role of OB ErbB4 in olfactory behaviors, we conducted a habituation/dishabituation test to assess the spontaneous behavioral responses of mice to repeatedly presented odors in the absence of any reward (Figure 1H). Both AAV-GFP mice and AAV-Cre-GFP mice habituated to four successive odor presentations with 2-min intertrial intervals: their behavioral responses to the same odor (isoamyl acetate) were progressively reduced. AAV-GFP mice rapidly dishabituated and increased their investigation time when a novel odor was presented (limonene, fifth trial), whereas AAV-Cre-GFP mice did not significantly alter their behavioral responses to the novel odor. Additionally, olfactory detection thresholds for isoamyl acetate and limonene were about 10^{-5} in AAV-GFP mice, but 10^{-4} in AAV-Cre-GFP mice (Figures 1I and 1J). In the habituation and dishabituation tasks, the two odors were used at levels far above the thresholds.

These results demonstrate that ErbB4 in the OB contributes to olfactory discrimination and dishabituation, but not to olfactory memory and habituation.

ErbB4 is expressed in PV interneurons within the EPL and IPL layers of the OB

RNAscope analysis was performed to detect mRNA levels of ErbB4 in the OB of PV-*Erbb4*$^{+/+}$ mice (generated by crossing PV-Cre mice with *loxP*-flanked *Erbb4* mice). As shown in Figures 2A and 2D, ErbB4 mRNA was expressed in all five layers of the OB (green); ErbB4-expressing neurons accounted for 58.0%, 44.4%, 71.9%, 69.8%, and 71.2% of the total cells (DAPI, blue) in the GL, EPL, MCL, IPL, and GCL. PV

interneurons (red) in the OB were mainly distributed in the EPL and IPL layers (Figure 2A); in these layers, ErbB4 tended to be located within PV interneurons (yellow) (Figures 2A-2C). Of the PV interneurons in the EPL and IPL, 69.4% and 100% expressed ErbB4, respectively (Figure 2E). Overall, ErbB4 expression in PV interneurons is highest within the IPL and lower within the EPL.

To further ascertain ErbB4 mRNA expression in individual PV interneurons, we conducted patch-clamp electrophysiology followed by single-cell analysis of reverse transcription-polymerase chain reaction (RT-PCR). For this experiment, we used slices from mice in which ErbB4 was specifically knocked out in PV interneurons (PV-Cre mice crossed with *loxP*-flanked *Erbb4* mice; PV-*Erbb4*$^{-/-}$) and their control littermates (PV-*Erbb4*$^{+/+}$). In addition to their morphology, size, and location, MCs were characterized electrophysiologically as having low-frequency spike discharges (<100 Hz), whereas PV interneurons were identified by their high-frequency spike discharges (>100 Hz) (Figure 2F). The cytosolic contents from identified MCs and PV neurons were then subjected to RT-PCR, showing that ErbB4 mRNA was only present in PV interneurons from PV-*Erbb4*$^{+/+}$ OB, but not in any of the MCs or PV interneurons from PV-*Erbb4*$^{-/-}$ OB (Figure 2G).

Next, ErbB4 proteins were detected by immunofluorescence and western blot analysis. Within the EPL of PV-*Erbb4*$^{+/+}$ mice, ErbB4 immunoreactivity was present in PV interneurons; this was not observed in the EPL of PV-*Erbb4*$^{-/-}$ mice (Figure 2H). Western blots showed that the level of ErbB4 protein was largely reduced, but not abolished, in the OB, prefrontal cortex, and hippocampus of PV-*Erbb4*$^{-/-}$ mice compared with their control littermates (Figures 2I and 2J). This suggests that ErbB4

is largely expressed in OB PV interneurons. Interestingly, ErbB4 deletion in the OB occurred at least two weeks prior to that in the prefrontal cortex and hippocampus (Figures 2I and 2J). Nissl staining showed that the overall lamina structures of the OB were not affected in PV-*Erbb4*$^{-/-}$ mice (Figure supplement 1).

The above results demonstrate strong expression of ErbB4 in PV fast-spiking interneurons within the EPL and IPL layers of the OB.

ErbB4 in PV interneurons is necessary for olfactory behaviors

To determine the contribution of ErbB4 expressed in PV interneurons to olfactory behaviors, we conducted reinforced go/no-go tests of olfactory discrimination in PV-*Erbb4*$^{+/+}$ and PV-*Erbb4*$^{-/-}$ mice. Both groups learned to distinguish simple odor pairs with an accuracy of more than 90% within 600 trials (Figure 3A); for the difficult odor pairs, PV-*Erbb4*$^{-/-}$ mice exhibited significantly lower accuracy than their control littermates (Figure 3A). During habituation/dishabituation tasks, both groups of mice habituated to the successive presentation of a familiar odor (isoamyl acetate, fourth trial) (Figure 3B); when a novel odor (limonene, fifth trial) was presented, PV-*Erbb4*$^{-/-}$ mice did not increase their investigation time, unlike their control littermates (Figure 3B). Similarly, when the presentation of the odors was reversed, the PV-*Erbb4*$^{-/-}$ group showed impaired dishabituation but the PV-*Erbb4*$^{+/+}$ group did not (Figure 3C). The olfactory detection thresholds for isoamyl acetate and limonene were slightly higher in PV-*Erbb4*$^{-/-}$ mice than in PV-*Erbb4*$^{+/+}$ mice (Figures 3D and 3E). We also tested mice with the buried food test, which depends on the animal's natural tendency to utilize olfactory cues for foraging, to confirm whether the mice could

smell volatile odors. As shown in Figure 3F, both PV-*Erbb4*$^{+/+}$ and PV-*Erbb4*$^{-/-}$ mice found the buried pellets rapidly over three consecutive testing days. On trial day 4, they also easily located the pellet within a similar timeframe when the food pellet was made visible by placing it on the surface, suggesting that selective deletion of ErbB4 in PV interneurons does not alter the ability of mice to detect and locate odorous sources.

These findings reveal that ErbB4 signaling in PV interneurons serves as an essential regulator of olfactory discrimination and dishabituation behaviors.

ErbB4 deletion in PV interneurons decreases odor-evoked responses in M/TCs in the OB

To investigate whether ErbB4 in PV interneurons modulates the activity of M/TCs, we recorded extracellular single-unit spontaneous and odor-evoked activity from M/TCs in the OB via tetrodes implanted in awake, head-fixed PV-*Erbb4*$^{-/-}$ and PV-*Erbb4*$^{+/+}$mice (Figure 4A). Single units were identified by principal-component analysis scan clustering of the spikes. Both excitatory (upper) and inhibitory (lower) responses were observed upon odor presentation from an odor delivery system (Figures 4B and 4C). Figure 4D shows the heat maps of odor-evoked firing rates of 240 M/TCs in PV-*Erbb4*$^{+/+}$ mice (left) and 232 M/TCs in PV-*Erbb4*$^{-/-}$ mice (right). As shown in Figure 4E, spontaneous firing in M/TCs was similar in PV-*Erbb4*$^{+/+}$ and PV-*Erbb4*$^{-/-}$ mice. However, odor-evoked firing of M/TCs was reduced by ErbB4 deletion in PV interneurons. Both the absolute value of odor-evoked changes and the normalized signal-to-noise ratio (SNR) were lower in PV-*Erbb4*$^{-/-}$ mice than PV-

222 *Erbb4*$^{+/+}$ mice. We next analyzed the differences in neurons with excitatory
223 responses, inhibitory responses, and no responses to odor presentation in PV-*Erbb4*$^{+/+}$
224 and PV-*Erbb4*$^{-/-}$ mice. Figure 4F and 4G show the changes in firing for excitatory
225 responses during odor presentation. Compared with the PV-*Erbb4*$^{+/+}$ group, M/TCs in
226 PV-*Erbb4*$^{-/-}$ mice had significantly higher spontaneous firing. The odor-evoked firing
227 rate was not changed by ErbB4 deletion in PV interneurons. The odor-evoked change
228 in mean firing rate (ΔMFR), SNR, and normalized SNR were all decreased in PV-
229 *Erbb4*$^{-/-}$ mice. Figures 4H and 4I showed the changes in firing for inhibitory
230 responses during odor presentation. Compared with the PV-*Erbb4*$^{+/+}$ group, M/TCs in
231 PV-*Erbb4*$^{-/-}$ mice had significantly lower spontaneous firing rates. The odor-evoked
232 firing rate of M/TCs was also decreased by ErbB4 deletion in PV interneurons.
233 Interestingly, the odor-evoked ΔMFR also showed a significant decrement in PV-
234 *Erbb4*$^{-/-}$ mice. Although the SNR was decreased, the normalized SNR was increased
235 in PV-*Erbb4*$^{-/-}$ mice. Figures 4J and 4K show the changes in firing for cells classes as
236 having no response during odor presentation. Spontaneous firing in M/TCs was higher
237 in PV-*Erbb4*$^{-/-}$ mice than in PV-*Erbb4*$^{+/+}$ mice. The odor-evoked firing rate of
238 M/TCs was also increased by ErbB4 deletion in PV interneurons. The absolute value
239 of odor-evoked changes (ΔMFR) and normalized SNR were not changed in PV-
240 *Erbb4*$^{-/-}$ mice compared with PV-*Erbb4*$^{+/+}$ mice. These results show that odor-
241 evoked excitatory and inhibitory responses were both compromised in PV-*Erbb4*$^{-/-}$
242 mice. In other words, the absolute change in firing rate (ΔMFR) was reduced after
243 ErbB4 ablation in PV interneurons regardless of the direction of the odor-evoked
244 change. Furthermore, the distribution of types of odor-evoked responses was

significantly altered in PV-*Erbb4*$^{-/-}$ mice compared with PV-*Erbb4*$^{+/+}$ mice (Figure 4L), suggesting compromised odor detection.

These results demonstrate that ErbB4 signaling in PV interneurons contributes to response intensity in M/TCs.

ErbB4 deletion in PV interneurons increases the power in the ongoing oscillations

Next, we investigated whether ErbB4 deletion in PV interneurons affects OB LFP oscillations. Raw LFP signals were divided into different frequency bands: theta (2 to 12 Hz), beta (15 to 35 Hz), low gamma (36 to 65 Hz), and high gamma (66 to 95 Hz) (Figure 5A). For all frequency bands of the ongoing baseline LFP, the power was higher in PV-*Erbb4*$^{-/-}$ mice than in PV-*Erbb4*$^{+/+}$ mice (Figures 5B-5I). These results suggest that ErbB4 in PV interneurons controls the spontaneous ongoing network oscillations in the OB.

ErbB4 in PV interneurons regulates the OB output

To further evaluate the physiological role of PV interneuron ErbB4 in the regulation of the OB output, we recorded spontaneous and evoked excitatory firing rates from principle MCs (the output cells) in OB slices. Cell-attached recordings showed that the frequency of MC spontaneous action potentials (sAPs) was dramatically enhanced in PV-*Erbb4*$^{-/-}$ mice compared with PV-*Erbb4*$^{+/+}$ mice (Figures 6A and 6B). Furthermore, the frequency of evoked action potentials (eAPs) was also higher in PV-*Erbb4*$^{-/-}$ mice (Figures 6A and 6B). However, the ratio of eAP to sAP firing (i.e., the

signal-to-noise ratio, SNR) was significantly lower in PV-*Erbb4*$^{-/-}$ mice (Figures 6A and 6B). More importantly, the SNR in response to stimuli at various intensities was lower in PV-*Erbb4*$^{-/-}$ mice than in their control littermates (Figures 6C and 6D), even though the overall current-evoked activity was elevated in PV-*Erbb4*$^{-/-}$ mice compared with their control littermates (Figures 6E and 6F).

These data indicate an involvement of PV interneuron ErbB4 in OB odor information processing.

ErbB4 in PV interneurons maintains inhibitory circuit plasticity in the OB

To investigate the neural mechanisms underlying the observed hyperexcitability in PV-*Erbb4*$^{-/-}$ mouse MCs, we tested whether the effect of ErbB4 deletion in PV interneurons could be occluded by GABAergic blockage. Bicuculline (10 μM), a selective $GABA_A$ receptor antagonist, elevated sAP firing in MCs from PV-*Erbb4*$^{+/+}$ mice but did not further increase MC hyperexcitability in PV-*Erbb4*$^{-/-}$ mice (Figures 7A and 7B). Bicuculline also reduced the eAP:sAP ratio in PV-*Erbb4*$^{+/+}$ MCs but not in PV-*Erbb4*$^{-/-}$ MCs (Figure 7A and 7B). These data indicate that the hyperexcitability in PV-*Erbb4*$^{-/-}$ MCs depends on GABAergic downregulation. To further dissect the role of GABAergic transmission in MC hyperexcitability in PV-*Erbb4*$^{-/-}$ mice, we recorded $GABA_A$ receptor-mediated miniature inhibitory postsynaptic currents (mIPSCs) from MCs in whole-cell mode. As shown in Figures 7C and 7D, the mIPSC frequency, but not the amplitude, were significantly reduced in PV-*Erbb4*$^{-/-}$ mice, suggesting a presynaptic impairment of inhibitory transmission. By contrast, neither the frequency nor the amplitude of glutamatergic receptor-

291 mediated miniature excitatory postsynaptic currents (mEPSCs) were altered in PV-
292 *Erbb4*$^{-/-}$ mice compared with their control littermates (Figures 7E and 7F). These
293 results suggest that ErbB4 in PV interneurons regulates olfactory information output
294 by maintenance of GABAergic inputs to MCs in the OB.

295 We next examined the influence of specific PV-ErbB4 knock-out on recurrent
296 inhibition of MCs. The prolonged hyperpolarization after AP firing that results from
297 recurrent inhibition was recorded in the whole-cell patch-clamping configuration.
298 Although the strength of recurrent inhibition is reported to depend on MC firing rates
299 (Margrie et al., 2001), neither the amplitude nor the decay times of recurrent
300 inhibitory postsynaptic potentials (IPSPs) showed significant alteration with the
301 increment in AP frequency in PV-*Erbb4*$^{-/-}$ mice (Figures 8A and 8B). One possible
302 explanation is that the loss of ErbB4 in PV interneurons severely impedes recurrent
303 IPSPs. To test this hypothesis, we recorded recurrent IPSPs after a given frequency of
304 APs in PV-*Erbb4*$^{-/-}$ mice. As shown in Figure 8C, a current injection of 80 pA in PV-
305 *Erbb4*$^{-/-}$ mice elicited approximately the same firing rate as a current injection of 100
306 pA in PV-*Erbb4*$^{+/+}$ mice. Remarkably, both the amplitude and the decay time constant
307 of the subsequent recurrent IPSP were reduced in PV-*Erbb4*$^{-/-}$ mice (Figure 8D).
308 Thus, ErbB4 ablation in PV interneurons weakens recurrent inhibition evoked by the
309 same MC firing intensity.

310 In addition, we attempted to isolate the broader lateral inhibition between MCs and
311 detect the effect of PV interneuron ErbB4 ablation on it. The recorded MC
312 corresponding to the target glomerulus was identified visually by Alexa 488 dye in the
313 whole-cell patch pipette, as described previously (Hu et al., 2017). A stimulating

electrode was then placed into the glomerulus located 3–4 glomeruli (~ 400 μm) caudally from the target glomerulus. Before recording, we sectioned through the glomerular layer (GL) and GC layer (GCL) between the sites of conditioning stimulation and the target glomerulus to eliminate interglomerular and GC-mediated lateral inhibition (Figure 8E). As shown in Figures 8F and 8G, a burst of conditioning stimuli (40 pulses at 100 Hz) caused a reduction in the MC firing rate in PV-*Erbb4*$^{+/+}$ mice, but not in PV-*Erbb4*$^{-/-}$ mice. When we also cut through the EPL, this broader lateral inhibition was totally abolished in PV-*Erbb4*$^{+/+}$ mice (Figures 8H-8J), indicating that the broader lateral inhibition was mediated by ErbB4-expressing PV interneurons.

The above data reveal an important contribution of PV interneuron ErbB4 to inhibitory circuit plasticity in the OB.

ErbB4 in OB PV interneurons is critical for olfactory behaviors

Finally, we evaluated the contribution of PV-ErbB4 in the OB to olfactory behaviors. ErbB4 expression in PV interneurons in the OB was reduced after *loxP*-flanked *Erbb4* mice were stereotactically injected in the OB with AAV-PV-Cre-GFP recombinants, but not when they were injected with the control AAV-PV-GFP group (Figures 9A and 9B). ErbB4 reduction in OB PV interneurons impaired behavioral discrimination of complex odor mixtures in the reinforced go/no-go task (Figure 9C). Spontaneous dishabituation responses were also impaired in AAV-PV-Cre-GFP mice compared with the control AAV-PV-GFP group (Figures 9D and 9E). Olfactory detection

thresholds for isoamyl acetate and limonene showed a slight increase in AAV-PV-Cre-GFP mice compared with AAV-PV-GFP mice (Figures 9F and 9G).

Thus, ErbB4 signaling in PV interneurons in the OB is required for olfactory discrimination and dishabituation in mice.

Discussion

ErbB4 expression is confined to PV interneurons in the cortex, hippocampus, basal ganglia, and amygdala (Bean et al., 2014; Fazzari et al., 2010) and plays a role in mood (Chen et al., 2022; Chung et al., 2018; Y. Huang et al., 2021) and cognitive behaviors (Cai and Shuman, 2022; Dominguez et al., 2019; Xu et al., 2022). In the present study, we explore the distribution of ErbB4 in OB microcircuits and its functional properties in olfactory behaviors. We found ErbB4-positive neurons were distributed in all layers of the OB and were co-localized with PV interneurons in the IPL and EPL layers. Furthermore, ErbB4 in PV interneurons regulated OB oscillations and odor-evoked output by maintaining GABA release and lateral and recurrent inhibition of MCs. ErbB4 deficiency either in the OB as a whole or specifically in PV interneurons impairs olfactory behavioral performance. Our findings provide evidence that PV interneurons regulate the plasticity of MC inhibitory neurotransmission and broad ongoing fluctuations—and hence olfactory behaviors of discrimination and dishabituation—via ErbB4 signaling.

We found that in the EPL and IPL, ErbB4 mRNA and proteins are mainly distributed in PV interneurons. Our data do not rule out ErbB4 expression in other subtypes of interneurons, as suggested by other studies in which ErbB4-positive cells were located

in all layers of the OB, including in PV interneurons (Bean et al., 2014; Bovetti et al., 2006; Neddens et al., 2011; Tan et al., 2022). ErbB4-deficiency in astrocytes and neural precursors alters the distribution and differentiation of interneurons in the mature OB and olfactory ability (Anton et al., 2004; Moy et al., 2009). Our data show that ErbB4 deficiency in PV interneurons does not affect the overall lamina structure of the OB. This may be because ErbB4 regulation of interneuron migration relies on a critical time window (prior to E13.5) (Batista-Brito et al., 2023; Mei and Nave, 2014). Our late-onset mutation of ErbB4 in the OB may not be early enough to cause the developmental phenotypes observed with early gene deletion.

In the OB, PV interneurons are excited predominantly by Ca^{2+}-permeable GluA2-lacking AMPA receptors and provide strong inhibitory input onto MCs (Kato et al., 2013). We recently found that ErbB4 is responsible for excitatory synaptic plasticity of AMPA receptors in the prefrontal cortex PV interneurons and olfactory associative reversal learning (Cai and Shuman, 2022; Xu et al., 2022). Furthermore, transsynaptic binding of ErbB4–Neurexin1β is critical for the activation of ErbB4 and extracellular signal-regulated kinase (ERK) 1/2 in PV interneurons, as well as brain-derived neurotrophic factor (BDNF) (Cai and Shuman, 2022; Xu et al., 2022). In the present study, ErbB4 activity in the OB was increased after odor discrimination. ErbB4 deficiency in PV interneurons impaired odor discrimination and dishabituation. These findings reveal that ErbB4 in the OB is sufficient and necessary for proper olfactory function. Neurexin1β is highly expressed in M/TCs and regulates inhibitory synaptic transmission in the OB (Wang et al., 2021). At the molecular level, we speculate that the association of ErbB4 with Neurexin1β may facilitate the formation of GluA2-

382 lacking AMPA receptor synapses onto OB PV interneurons, thus regulating GABA
383 release. Thus, an NRXN1β-ErbB4-ERK1/2-BDNF pathway may underlie olfactory
384 processes.

385 The anatomical and physiological properties of the circuit between PV interneurons
386 and M/TCs in the OB have been well characterized (Huang et al., 2013; Kato et al.,
387 2013; Matsuno et al., 2017; Miyamichi et al., 2013). PV interneurons exert linear and
388 robust inhibition onto MCs, which is hypothesized to effectively change the odor
389 response threshold of the MCs (Kato et al., 2013; Miyamichi et al., 2013). We report
390 here that knock-out of ErbB4 in PV interneurons decreased inhibition onto MCs and
391 increased electrical-stimulation-elicited activity of MCs *in vitro*. However, the
392 increment in spontaneous activity was larger than the increase in olfactory-nerve-
393 evoked activity, indicating enhancement of background noise. Furthermore, ErbB4
394 deletion in PV interneurons decreased odor-evoked firing in M/TCs and changed the
395 SNR *in vivo*. Thus, loss of ErbB4 in PV interneurons most likely elevates the odor
396 detection threshold by interfering with the SNR in the OB. In the neocortex, PV
397 interneurons have been implicated in the formation of gamma oscillations to reduce
398 circuit noise and amplify circuit signals (Sohal et al., 2009). For example, selective
399 deletion of ErbB4 from fast-spiking PV interneurons increases gamma oscillations in
400 the hippocampus (Del Pino et al., 2013). Gamma oscillations in the OB are critical for
401 odor discrimination (Lepousez and Lledo, 2013) and the generation of gamma
402 rhythms in the OB requires inhibitory synaptic transmission in the EPL (Lagier et al.,
403 2004; Lepousez and Lledo, 2013). Although PV interneurons in the EPL show broad
404 tuning to odors, ErbB4 deficiency in PV interneurons may disrupt gamma rhythms by

reducing inhibitory synaptic transmission in the OB, leading to the impaired odor discrimination observed in the present study. Odor detection is a prerequisite of discrimination, so the impaired discrimination could also be due to the elevated detection threshold. Also, ErbB4 in PV interneurons broadly regulate olfactory-related functions through tuning all frequency bands of ongoing oscillations in the OB. Overall, our study reveals the functions of PV interneurons in the OB and the associated molecular mechanisms underlying the observed olfactory behaviors.

The spontaneous activity of MCs is driven mainly by inhibitory interneurons rather than excitatory olfactory sensory neurons (Duchamp-Viret and Duchamp, 1993; Hu et al., 2021; Hu et al., 2017; Stakic et al., 2011). GCs are the most prominent GABAergic interneuron type and established play an established central role in the precise regulation of MC firing rates and synchrony through lateral and recurrent inhibitory mechanisms (Aghvami et al., 2022). PV interneurons also form reciprocal synapses with MCs but provide strong, relatively broadly tuned inhibition and linearly control OB output (Huang et al., 2013; Kato et al., 2013; Matsuno et al., 2017; Miyamichi et al., 2013). Here we observed decreases in mIPSC frequency, recurrent inhibition, and lateral inhibition in PV-*Erbb4*$^{-/-}$ mice, suggesting that ErbB4 in PV interneurons plays an important role in regulating M/TC activity through recurrent and lateral inhibitory mechanisms. Another interesting finding is that the changes in spontaneous firing rate differed for odor-evoked excitatory and inhibitory responses *in vivo*, suggesting that ErbB4 in PV interneurons dynamically regulates the spontaneous activity of M/TCs in response to different odors. Our findings help unravel the circuit

and molecular mechanisms underlying the modulatory effect of PV interneurons on M/TC odor responses in awake mice.

In the present study, we identified a spatially broad (300–400 μm in our experiments) lateral inhibitory circuit in the OB, requiring ErbB4 in PV interneurons. Thus, our findings raise the possibility that distinct classes of interneurons mediate interglomerular lateral inhibition within discrepant spatial dimensions. For example, short-axon cells usually have relatively long axonal arbors, spanning as much as 1000 μm (Aungst et al., 2003), that may effectively mediate the broadest (up to 600 μm) spatial dimension of lateral inhibition (Whitesell et al., 2013). By contrast, GCs connect only to their neighboring MCs (< 50–100 μm in distance); their lateral inhibition is narrowly tuned (Miyamichi et al., 2013). And here we found that PV interneurons mediate spatially moderate (300–400 μm) lateral inhibition via ErbB4. Future studies would benefit by defining the functions (e.g., contrast enhancement) of these distinct spatial dimensions of lateral inhibition in odor information processing. Both recurrent and lateral inhibition implement a “winner-takes-all” mechanism: Once the strongest input excites the principal cells, firings in the remaining inputs is inhibited (de Almeida et al., 2009; Espinoza et al., 2018; Karmakar and Sarkar, 2013). The regulation of recurrent and lateral inhibition by ErbB4 sheds light on how PV interneurons mediate this “winner-takes-all” inhibitory microcircuit.

In recent decades, impaired odor discrimination has been found in the earliest stages of neurodegenerative disorders, such as Alzheimer’s disease and Parkinson’s disease (Dan et al., 2021). Olfactory discrimination deficits develop prior to neuropsychological characteristics in patients with Alzheimer’s disease and have

450 significant predictive valence for future cognitive decline in healthy aging individuals
451 (Carnemolla et al., 2020; Sohrabi et al., 2012; Uchida et al., 2020; Yoshitake et al.,
452 2022). *ErbB4* has been independently identified as a susceptibility gene for
453 Alzheimer's disease and schizophrenia (Mei and Nave, 2014; Swaminathan et al.,
454 2012). Here we showed that selective loss of ErbB4 in OB PV interneurons impairs
455 olfactory behaviors. Although ongoing fluctuation status predict behavioral alteration
456 in heath and disease, the contributions of its different frequency bands are less known
457 (Iemi et al., 2022). This study links the broad ongoing fluctuations with OB output
458 and hence controlling rapid recovery from olfactory adaptation and complex odor
459 discrimination. Our findings will help us to better understanding the molecular and
460 cellular mechanisms behind early olfactory dysfunction in neurodegenerative disease
461 and schizophrenia and provide a potential strategy for treatment.

462 In summary, our study demonstrates a crucial role of ErbB4 in olfactory behaviors,
463 involving ErbB4-expressing PV interneurons. Our results are most likely attributable
464 to presynaptic impairments in the inhibitory microcircuits in the OB, which affect the
465 processing of odor information and results in abnormal output signals from the
466 M/TCs. The data presented may be promising not only for understanding
467 physiological functions of ErbB4 in the OB but also for providing early diagnosis in
468 Alzheimer's disease and schizophrenia and potential therapeutic applications,
469 particularly in patients with olfactory disorders.
470

471 **Materials and methods**

472 **Reagents and animals**

Rabbit polyclonal anti-ErbB4 antibody (sc-283, 1:2000, or 1:1000 for blotting, 1:100 for staining) was purchased from Santa Cruz, mouse monoclonal anti-PV antibody (P3088, 1:7000 for staining) was purchased from Sigma, anti-β-actin antibody (4970, 1:5000 for blotting) was purchased from Cell Signaling Technology, goat anti-rabbit IgG conjugated with Alexa Fluor 488 (A11089, 1:400 for staining) and goat anti-mouse IgG conjugated with Alexa Fluor 594 (A11037, 1:400 for staining) were purchased from Invitrogen. The Na^+ channel blocker tetrodotoxin (TTX, 1069) was purchased from Tocris and $GABA_A$ receptor antagonist (+)-bicuculline (ALX-550-515) from Enzo. Other chemicals were sourced from Merck Sigma–Aldrich.

ErbB4 conditional knockout mice (PV-*Erbb4*$^{-/-}$) were generated by crossing PV-Cre mice with *loxP*-flanked *Erbb4* mice (Garcia-Rivello et al., 2005; Wen et al., 2010; Xu et al., 2022). Mice were housed under a 12-h light/dark cycle and had ad libitum access to water and food except for during the behavioral tests. All experiments were done with both sexes of mice. All experimental procedures were conducted in accordance with the guidelines described in the revised Regulations for the Administration of Affairs Concerning Experimental Animals (2011) in China and approved by the local Institutional Animal Care and Use Committee.

Western blot analysis

Tissues were homogenized in the homogenization buffer containing 50 mM Mops (pH 7.4), 320 mM sucrose, 100 mM KCl, 0.5 mM $MgCl_2$, 0.2 mM dithiothreitol, and phosphatase and protease inhibitors (20 mM sodium pyrophosphate, 20 mM β-glycerophosphate, 50 mM NaF, 1 mM each of EDTA and EGTA, sodium orthovanadate, p-nitrophenyl phosphate, PMSF and benzamidine, and 5 μg/ml each of

aprotinin, leupeptin, and pepstatin A). Homogenates (40 μg of protein) were resolved by 7.5% SDS/PAGE and transferred to nitrocellulose membranes (Millipore), which were blocked with 3% bovine serum albumin (BSA) in Washing buffer (100 mM NaCl, 0.1% Tween-100, 10 mM Tris, pH 7.5). Membranes were incubated overnight with the primary antibody in 1% BSA then probed with the secondary antibody for 1 h. Signals were visualized with Immobilon Western Chemiluminescent HRP Substrate. Immunobloting quantification was performed in ImageJ (NIH).

Virus injections

Virus injections were performed as previously described (Kato et al., 2013). Neonatal *loxP*-flanked ErbB4 mice (0–3 days old) were cryo-anesthetized in an ice-cold chamber and head-fixed in a stereotaxic device (RWD68513, RWD Life Science). Virus (AAV-Cre-GFP [55764361#] and AAV-GFP [55764363#] were from Obio Technology; AAV-PV-Cre-GFP [PT-0275] and AAV-Cre-GFP [PT-0154] were from BrainVTA) was delivered with titer 10^{12} genome copies per milliliter. The injection coordinates were as follows (anteroposterior, mediolateral, and dorsoventral relative to the intersection of the midline and the inferior cerebral vein, in mm): (+0.2, ±0.3, −0.9/0.7), (+0.2, ±0.6, −0.9/0.7), (+0.5, ±0.3, −0.7/0.5) and (+0.5, ±0.6, −0.7/0.5). Virus (20 nl per site) were injected with a speed of 23 nl/s through a glass pipette. Behavioral tests were performed 10 weeks after virus injections.

Behavioral analysis

Behavioral analysis was carried out by investigators unaware of the genotypes of the experimental animals, to ensure that the study was blinded.

Go/no-go test

519 Mice were deprived of water then explored the set-up (Thinkerbiotech) and learned to
520 lick a metal tube just below the odor port to obtain water, as previously described (Hu
521 et al., 2021). Mice self-initiated trials by poking their nose into the sampling port,
522 which interrupted an infrared light beam. All odors were diluted to 0.01% in mineral
523 oil and further diluted 1:20 in air in the olfactometers. Odor pairs were presented for 2
524 s in a randomized order 0.5 s after trial initiation. Prior to training on the go/no-go
525 task, mice were trained to perform a go/go task for two days. During the go/go task, if
526 the mice licked within the odor presentation period when presented with either of the
527 odor pairs, they received the water reward. Then the go/no-go task was performed to
528 train mice to discriminate between a pair of odors to receive the water reward. Mice
529 learned to lick the metal tube to receive water in response to the rewarded odor (hit) at
530 the end of the trial (2.5-s total trial duration) and avoid licking the metal tube for the
531 unrewarded odor (correct rejection). Licking when presented with the unrewarded
532 odor (false alarm) led to no water reward and a timeout of up to 10 s. Performance
533 "accuracy" was calculated as the percentage of correct trials (number of hits and
534 correct rejections) over the total number of trials. The weight of mice under water
535 restriction was strictly monitored (>85% of the initial body weight) during the entire
536 test. Eight-channel semi-automated olfactometers, data acquisition, and analysis were
537 all controlled through computer programs written in LabVIEW and Matlab
538 (Thinkerbiotech).
539 Habituation/dishabituation test
540 Odors were presented by placing a cotton swab scented with an odor solution (150 μl,
541 1:2000, freshly prepared with mineral oil before each experiment) above the floor of

the animal's home cage, as previously described (Hu et al., 2021). Mice were familiarized with a first odor in four successive trials (habituation) and then exposed to a novel odor on the fifth trial (dishabituation). The five successive 2-min trials were separated by 2-min intervals. Each trial was videotaped, and the investigation time of the swab was quantified offline. Sniffing behavior was defined as whenever the mouse had its nose within a 1-cm radius from the surface of the cotton swab. Lack of novel odor discrimination was considered to occur when mice spent as much time investigating the swab during the dishabituation trial as in the fourth habituation trial.

Odor detection threshold test

Odor detection thresholds were obtained according to procedures described previously (Nicolis di Robilant et al., 2019). Odors (isoamyl acetate and limonene) were freshly diluted with mineral oil to different concentrations (10^{-6}, 10^{-5}, and 10^{-4} in 150 µl) and applied to a cotton swab, then placed above the floor of the animal's cage along with mineral oil on the other side. Each trial was conducted for 2 min separated by 2-min intertrial intervals. Mice were deemed able to detect the odor when they spent more time sniffing the diluted odor than the mineral oil, and the minimum concentration detected by mice was taken as the threshold.

Buried food pellet test

All chow pellets were removed from the home cage 24 h before testing. Mice had free access to water. Before each experimental trial, 10–12-week-old mice were familiarized with the test cage for 10 min. On each trial, a single mouse was placed in the test cage (46 × 30 × 16 cm) to recover a 0.2-g food pellet that was buried 0.5-cm below the surface of the bedding material. The location of the food pellet was selected

565 at random. The time between when the mouse was placed in the cage and when it
566 grasped the food pellet with its forepaws or teeth was recorded and defined as the
567 latency. Mice were allowed to consume the food pellet and were then returned to their
568 cages. The food pellet was presented to the mouse for consumption if not be found
569 within 300 s, in which case the latency was recorded as 300 s. A visible food pellet
570 test was conducted as a control: the procedures were identical except the pellet was
571 placed randomly on surface of the bedding.

572 **RNAscope**

573 To examine the expression of the ErbB4 gene, RNAscope was performed using an
574 RNAscope Fluorescent Multiplex Kit (Advanced Cell Diagnostics, ACDBio) per
575 manufacturer's recommendations. Briefly, the OB was cut into 20-μm coronal
576 sections. Sections were serially thaw-mounted onto 20 slides (Fisherbrand) through
577 the entire OB and then air-dried for 1 h at room temperature prior to storage at −80 ℃.
578 RNAscope probes were obtained from ACDBio for ErbB4 (Cat #318721) and PV
579 (Cat #421931-C2). Sections were counterstained for the nuclear marker DAPI
580 (Abcam, Cat #104139).

581 **Immunohistochemistry**

582 Mice (4 weeks old) were perfused transcardially with 4% paraformaldehyde.
583 Dissected brains were postfixed overnight and dehydrated in 30% sucrose for 24 h at
584 4 ℃, then the OBs were cut into 40-μm-thick sections horizontally on a vibratome
585 (Leica). OB sections were processed for double immunofluorescence in 0.1 M PBS
586 (pH 7.4) as follows: three washes in 0.1 M PBS, 10 min each; blocking in 0.1 M PBS
587 containing 10% normal goat serum, 1% BSA, and 0.25% Triton X-100 overnight at

4 ℃; primary antibody incubation for 40 h at 4 ℃ in blocking solution; three washes; secondary antibody for 90 min at room temperature in blocking solution; three washes; rinsing with PBS and mounting with a mounting medium (Vectashield, Vector Laboratories). Immunoreactivity was imaged by Alexa 488- and Alexa 594-conjugated goat anti-mouse IgG. Sections were analyzed with a confocal microscope (Zeiss L710) at 20× and 60× magnification. Cresyl violet staining was carried out as described previously. The OCT-embedded OBs were cut into 20-μm sections. OB sections were stained with 0.1% cresyl violet for 35 min, dehydrated with ascending grades of alcohol, then cleared with xylene, mounted, and observed under an Olympus microscope.

Slice preparation and patch-clamp electrophysiology

Acute OB slices were prepared as described previously (Hu et al., 2021; Hu et al., 2017). In brief, animals were heavily anesthetized with ketamine/xylazine (140/20 mg/kg, intraperitoneally) and decapitated, then the OB was quickly removed and immersed in ice-cold and oxygenated (95% O_2/5% CO_2) ACSF containing (in mM: 124 NaCl, 3 KCl, 2 $CaCl_2$ 1.3 $MgCl_2$, 25 $NaHCO_3$, 1.25 NaH_2PO_4, and 10 glucose). Horizontal slices (300 μm) were cut with a VT1000s vibratome (Leica), recovered at 37 ℃ for 30 min and then maintained at 25 ℃.

OB slices were transferred to the recording chamber, which was warmed to 33 ℃ and superfused with oxygenated ACSF (2 mL/min). Neurons were visualized with infrared optics using an upright microscope equipped with a 60× water-immersion lens (BX51WI, Olympus) and an infrared-sensitive CCD camera. MCs and PV interneurons were identified by their fluorescence, morphology, size, location, and

611 electrophysiological characterization.

612 For cell-attached mode, recording pipettes with resistances of 6–8 MΩ were pulled
613 from borosilicate glass capillaries (P97, Sutter Instrument Co) and filled with ACSF.
614 To record olfactory-nerve-evoked responses, monophasic square pulses (200 μs) with
615 an intensity of 0.2 mA or gradual increasing intensities of 0.1, 0.15, 0.2, 0.25, and 0.3
616 mA were delivered through a concentric electrode that was placed on the outermost
617 layer of the OB, near the recorded MC. The frequency of firing was defined as
618 number of action potentials/time window. A 5-s window prior to olfactory-nerve
619 stimulation was used to assess the spontaneous firing rate, and the first 1-s window
620 after stimulation of the olfactory nerve was used to assess the evoked response. The
621 signal-to-noise ratio (SNR) was defined as the ratio of evoked responses to
622 spontaneous firing in the 5 s prior to olfactory nerve stimulation.
623 To record whole-cell action potentials and mEPSCs, pipettes were filled with a
624 solution containing 140 mM K-methylsulfate, 4 mM NaCl, 10 mM HEPES, 0.2 mM
625 EGTA, 4 mM MgATP, 0.3 mM Na_3GTP, and 10 mM phosphocreatine (pH was
626 adjusted to 7.4 with KOH). MCs were held at −65 mV in the presence of 20 μM
627 bicuculline and 1 μM TTX during recording of mEPSC.
628 To isolate mIPSCs, MCs were held at −65 mV in the presence of 50 μM AP5, 20 μM
629 NBQX and 1 μM TTX with a CsCl-base intracellular solution containing (mM): 135
630 CsCl, 10 HEPES, 0.2 EGTA, 2 Na_2ATP, 0.3 Na_3GTP, and 10 glucose.
631 Miniature events in a 5-min recording period were analyzed in Mini Analysis
632 software (Synaptosoft, ver. 6.07).
633 Lateral inhibition recordings were obtained according to procedures described

previously (Arevian et al., 2008; Hu et al., 2017; Whitesell et al., 2013). Briefly, a concentric bipolar stimulating electrode was placed into the caudal glomerulus located 3–4 glomeruli (~400 μm) caudally from the one correlated with the recorded MC. The intensity of the stimulation was moderate (100 μA) to avoid stimulating the glomerulus of the recorded MC, which would invariably evoke excitatory postsynaptic potentials (EPSPs). Pulses of 200-μs duration were delivered at 100 Hz and synchronized with the recording by a Master-8 stimulator (AMPI). Alexa 488 dye (100 μM) was added to the intracellular solution to visualize cell morphology. Signals were acquired with a MultiClamp 700B amplifier (Molecular Devices), filtered at 2 kHz, and sampled at 10 kHz with a Digidata 1440A interface (Molecular Devices) and Clampex 10.2 software (Molecular Devices). Data were accepted when the series resistance stayed within 15% of the initial value. The spontaneous and miniature events in a 5-min recording period were analyzed in Mini Analysis software (Synaptosoft, ver. 6.07).

Single-cell RT-PCR

At the end of the recording session, gentle suction was applied to aspirate the cytoplasm into the pipette while maintaining a tight seal. After complete incorporation of the soma, the pipette was carefully removed from the neuron to perform an outside-out patch recording, then quickly removed from the bath. The harvested contents were expelled into a PCR tube. RT was performed in a final volume of 20 μl using a QuantiTect Reverse Transcription Kit (205311, Qiagen). Next, outer and nested primer sets were used for two consecutive rounds of PCR, as described previously (Vullhorst et al., 2009). For the first round of PCR, all targets were amplified

simultaneously using outer primer pairs for PV and ErbB4 (first set PCR primer sequences: GCCTGAAGAAAAAGAACCCG and AATCTTGCCGTCCCCATCCT for PV, CCAGCCCAGCGCTTCTCAGTCAG and GTATTTCGGTCAGGTTCTTTAATCC for ErbB4, 20 pmol of each) in a final volume of 100 μl for 21 cycles. Each cycle comprised a 94 ℃ denaturation for 30 s, 60 ℃ annealing for 30 s, and 72 ℃ extension for 30 s. For the second round of PCR, PV and ErbB4 were separately reamplified using specific nested primer sets for 35 cycles of PCR, as described above, with 2 μl of the first PCR product as template (second primer sequences: CGGATGAGGTGAAGAAGGTGT and TCCCCATCCTTGTCTCCAGC for PV, CTGACCTGGAACAGCAGTACCGA and AGGCATAGCGATCTTCATATAGT for ErbB4). Products were assayed on 2% agarose gels stained with Gel Red, with dl1000 as a molecular weight marker.

Implantation of electrodes for in vivo electrophysiological recordings

After being deeply anesthetized, mice were secured in a stereotaxic frame (RWD Instruments, Shenzhen, China). The fur on the surface of the scalp from the midpoint between the ears to the midline of the orbits was removed. For spike and LFP recording, tetrodes (single-wire diameter, item no. PF000591, RO-800, 0.0005”/12.7 μm, coating 1/4 hard PAC, Sandvik) were implanted into the hole drilled above the OB (4.28 mm anterior from bregma, 1.0 mm lateral from the midline). The tetrodes were lowered into the brain after the dura mater was punctured. Each tetrode was connected to a 16-channel electrode interface board (EIB-16, Neuralynx). Signals were sent to a headstage and amplified by a 16-channel amplifier (Plexon DigiAmp) and monitored in real time to ensure optimal placement within the ventral MC layer.

A stainless-steel screw was inserted into the parietal bone (1 mm posterior to bregma and 1 mm from the midline) and connected to the ground as the reference electrode. Finally, a custom-designed aluminum head plate was sealed to the skull surface with dental cement to enable head fixation during recordings. Mice were returned to their home cages to recover for at least a week.

Electrophysiological recordings in awake mice

Recordings began at least one week after electrode implantation. Mice were head-fixed with two horizontal bars fixed to the headplate by two screws and were able to walk on an air-supported floating polystyrene foam ball (Thinkerbiotech, Nanjing, China). For spike recordings, the signals from the tetrodes were filtered at 300–5000 Hz and sampled at 40 kHz. Odor stimulus event markers were recorded alongside the spike recording. For local field potential (LFP) recordings, the signals were amplified by 2000× gain, bandpass filtered at 0.1–300 Hz, and sampled at 1 kHz (Plexon DigiAmp).

Odor application for electrophysiological recordings in awake mice

A standardized panel of eight odors (isoamyl acetate, 2-heptanone, phenyl acetate, benzaldehyde, dimethylbutyric acid, n-heptane acid, n-pentanol, and 2-pentanone, purchased form Sinopharm Chemical Reagent) were presented by an odor delivery system (Thinkerbiotech). All odors were dissolved at 1% (v/v) dilution in mineral oil. During odor stimulation, a stream of charcoal-filtered air flowed over the oil at a total flow rate of 1 L/min and the vapour-phase concentration was further diluted to 1/20 by the carrier airflow. The mice were first anesthetized with isoflurane for about 20 s and then rapidly transferred to the polystyrene foam ball. Before the start of *in vivo*

electrophysiological recordings, mice were allowed 10 min to acclimate to the fixation and recover from the isoflurane. Odor presentation was controlled by the odor delivery system coupled to a solenoid valve driven by a digital-to-analog converter. Each odor was presented for 2 s and the inter-trial interval was about 24 s.

Off-line spike sorting and statistics of the unit data

Spikes were identified and sorted from the raw data in Offline Sorter V4 software (Plexon). Spikes were detected and sorted when they had an amplitude larger than five times the standard deviation of the noise. Principal component analysis of the data was performed to separate different units. When <0.75% of the inter-spike intervals were <1 ms, a unit was classified as a single unit. Data from 4 s before until 6 s after the onset of the odor stimulation event were extracted, and the spike firing rate was averaged within 100-ms bins to generate a peristimulus time histogram (PSTH). The spontaneous firing rate and the odor-evoked firing rate were calculated by averaging the spikes recorded during the periods 4–2 s before odor stimulation and 2 s after the start of odor stimulation, respectively. To determine whether the odor evoked a significant response, we used a Wilcoxon signed-rank test to compare the spontaneous (baseline) firing rate with the odor-evoked firing rate across all trials for each unit–odor pair. The particular unit–odor pair was defined as nonresponsive if the *P* value was >0.05. Conversely, if the *P* value was <0.05 the unit–odor pair was defined as responsive. The unit–odor pairs were further categorized as showing an excitatory response (if the odor-evoked firing rate was greater than the spontaneous firing rate) or an inhibitory response (if the odor-evoked firing rate was lower than the spontaneous firing rate). Subtracting the baseline firing rate from the odor-evoked

726 firing rate yielded the Δ mean firing rate (MFR). The normalized signal-to-noise ratio
727 (SNR) was defined as follows: normalized SNR = | (odor-evoked firing rate/the
728 baseline firing rate) −1|.

729 **LFP signal analysis**

730 LFP signals were analyzed with a Matlab script and divided into different frequency
731 bands: theta (2 to 12 Hz), beta (15 to 35 Hz), low gamma (36 to 65 Hz), and high
732 gamma (66 to 95 Hz). The spectral power of each frequency resolution was calculated
733 over 4-s window. The spectral power of all frequencies within the bandwidth was
734 averaged.

735 **Statistical analysis**

736 Data are presented as the mean ±standard error of the mean (SEM). Unpaired or paired
737 *t* tests were used to compare data from two groups. A Chi-square test was used for
738 comparison of two constituent ratios. One or two-way ANOVAs were used to for
739 comparisons between more than two groups (western blots, behavioral and
740 electrophysiological studies). All tests were two-sided and considered to be statistically
741 significant when $P < 0.05$. Statistical analyses were performed in GraphPad Prism.

742

743 **Additional information**

744 **Competing interest**

745 The authors declare no competing interests.

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Bin Hu, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing—original draft; Chi Geng, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft; Feng Guo, Ying Liu, Data curation, Formal analysis, Investigation, Methodology; Ran Wang, Investigation, Methodology; You-Ting Chen, Methodology; Xiao-Yu Hou, Conceptualization, Resources, Supervision, Funding acquisition, Validation, Project administration, Writing—review and editing.

Ethics

All experimental procedures were conducted in accordance with the guidelines described in the revised Regulations for the Administration of Affairs Concerning Experimental Animals (2011) in China and approved by the local Institutional Animal Care and Use Committee.

Additional files

Supplementary files

764 • **Supplementary file 1.** The overall lamina structure of the OB. Cresyl violet
765 staining was performed to examine coronal structure sections of the OB at 2 and 6
766 months of age.

767 **Data availability**

768 All data generated or analyzed during this study are included in the manuscript and
769 supporting files.

770

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Figure legends

Figure 1. ErbB4 in the OB is critical for odor discrimination and dishabituation. (A) Behavioral paradigm for olfactory associative learning without odor discrimination (go/go task). Mice inserted their snout into the sampling port to trigger odors. The schematic describes the timeline of a single trial. Mice learned to lick the metal tube to receive water in response to either of the odors in the pair (reward, hit). (B) Timeline for a single trial in the go/no-go odor discrimination task. Mice learned to avoid licking the metal tube for the unrewarded odor (correct rejection, CR). Licking when presented with the unrewarded odor (false alarm, FA) led to no water reward and a timeout of up to 10 s. (C and D) ErbB4 activity in the OB was elevated after training on the reinforced go/no-go odor discrimination task. Relative p-ErbB4 and ErbB4 levels were normalized to their respective β-actin control groups in the western blot analysis (n = 3 mice per group, $t_{(2)}$ = 4.34, P = 0.0492, paired t test). (E) Schema indicating virus injection sites. To specifically delete ErbB4 protein in the OB, AAV-Cre-GFP was injected into bilateral OB of neonatal *loxP*-flanked ErbB4 mice. (F) Reduced ErbB4 expression in the OB of a mouse injected with AAV-Cre-GFP. (G) Odor discrimination performance under the reinforced go/no-go task. The accuracy for simple odor pairs was similar for the control and experimental groups (n = 8 mice per group, $F_{(1, 14)}$ = 2.83, P = 0.1148, two-way ANOVA). However, the accuracy for difficult odor mixtures (6/4 V 4/6) was reduced in AAV-Cre-GFP mice ($F_{(1, 14)}$ = 16.14, P = 0.0013, two-way ANOVA). (H) Odor performance under a spontaneous habituation/dishabituation task. Both animal groups showed a decline in investigation time to isoamyl acetate over the habituation period (n = 9 AAV-GFP

mice, $P < 0.0001$; $n = 10$ AAV-Cre-GFP mice, $P < 0.0001$, $F_{(3, 51)} = 21.83$, two-way ANOVA). However, AAV-GFP ($F_{(1, 17)} = 3.52$, $P = 0.0065$), but not AAV-Cre-GFP mice ($P = 0.6296$, two-way ANOVA), showed an increase in investigation time toward limonene in the dishabituation period. (I) Odor detection threshold to isoamyl acetate. For AAV-GFP mice, the sniffing time toward isoamyl acetate was significantly higher than that for mineral oil at concentrations of 10^{-5} and 10^{-4}, but not 10^{-6}. (n = 12 mice, $F_{(1, 21)} = 0.19$, 4.18 and 14.88, $P = 0.5689$, 0.0111, and 0.0090, two-way ANOVA). These results show that AAV-GFP mice were able to detect isoamyl acetate at a concentration of 10^{-5}. For AAV-Cre-GFP mice, the sniffing time toward isoamyl acetate was significantly higher than that for mineral oil at a concentration of 10^{-4}, but not at concentrations of 10^{-5} and 10^{-6} (n = 11 mice, $P =$ 0.9672, 0.8713, and 0.0172, two-way ANOVA). These results show that AAV-Cre-GFP mice only detect isoamyl acetate at a concentration of 10^{-4}. (J) Similarly, AAV-GFP mice were able to detect limonene at a concentration of 10^{-5} (n = 12 mice, $F_{(1, 21)} = 0.03$, 4.77 and 12.96, $P = 0.9603$, 0.0011, and 0.0069, two-way ANOVA), whereas AAV-Cre-GFP mice only detected limonene at a concentration of 10^{-4} (n = 11 mice, $P = 0.7671$, 0.5490, and 0.0463, two-way ANOVA). Data are presented as means ± s.e.m. * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$, n.s. = not significant. EPL, external plexiform layer; GCL, granule cell layer; GL, glomerular layer; IPL, internal plexiform layer; MCL, mitral cell layer.

Figure 2. ErbB4 proteins are largely expressed in PV interneurons in the OB. (A) *In vitro* imaging of ErbB4 mRNA in sections from PV-*Erbb4*$^{+/+}$ mice (generated by

crossing PV-Cre mice with *loxP*-flanked *Erbb4* mice). Double single-molecule fluorescence in situ hybridization of ErbB4 (green) and PV (red) in the OB. Scale bar, 200 μm. (B) Magnified view of the EPL box from A. Scale bar, 50 μm. (C) Magnified view of the IPL box from A. Scale bar, 50 μm. (D) Summarized data showing the proportion of ErbB4-expressing neurons in different layers (n = 24, 24, 24, 22, and 24 fields from 4 mice). (E) Summarized data showing the proportion of the ErbB4/PV double-positive neurons relative to the total number of PV interneurons in the EPL and IPL (*n* = 18 and 5 fields from 4 mice). DAPI staining was used to determine the total number of cells. (F) *In vitro* electrophysiology experiments performed in slices from PV-*Erbb4*$^{+/+}$ or PV-*Erbb4*$^{-/-}$ mice (generated by crossing PV-Cre mice with *loxP*-flanked *Erbb4* mice). Representative examples of action potentials (APs) elicited by positive current injection (500 ms, 300 pA), recorded from an MC (left) and a fast-spiking PV interneuron (right). (G) Corresponding single-cell RT-PCR analyses showing that ErbB4 mRNA is detected only in PV interneurons (PVN) from PV-*Erbb4*$^{+/+}$ OB. Dl1000 was used as the size reference (M, 300, 200 and 100 base-pair fragments are indicated). (H) Specific deletion of ErbB4 in EPL PV interneurons of the OB. OB sections from PV-*Erbb4*$^{+/+}$ and PV-*Erbb4*$^{-/-}$ mice (P28) were stained with DAPI, anti-PV and ErbB4 antibody. Scale bars represent 50 and 20 μm respectively. (I and J) Western blots showing that ErbB4 in PV-*Erbb4*$^{-/-}$ OB was largely reduced from P7 onward, whereas ErbB4 in the PFC and hippocampus began to decrease only at P21. Relative levels were normalized to their respective P7 groups of control littermates (*n* = 3 mice per group, OB: $F_{(1, 8)}$ = 245.70, P < 0.0001, P = 0.0002, 0.0006, and 0.0010; PFC: $F_{(1, 8)}$ = 61.20, P = 0.0532, 0.1791, 0.0075, and

0.0021; Hi: $F_{(1, 8)}$ = 38.25, P = 0.2585, 0.1005, 0.0139 and 0.0382, two-way ANOVA). Data are presented as means ± s.e.m. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.001, n.s. = not significant. EPL, external plexiform layer; GCL, granule cell layer; GL, glomerular layer; Hi, hippocampus; MCL, mitral cell layer; ONL, olfactory nerve layer; PFC, prefrontal cortex.

Figure 3. ErbB4 in PV interneurons is critical for olfactory behaviors. (A) Odor discrimination under the reinforced go/no-go task in PV-*Erbb4*$^{-/-}$ mice. The accuracy for simple odor pairs was indistinguishable (n = 5 and 6 mice, $F_{(1, 9)}$ = 0.70, P = 0.4260, two-way ANOVA). However, the accuracy for difficult odor pairs was significantly lower in PV-*Erbb4*$^{-/-}$ mice ($F_{(1, 9)}$ = 9.12, P = 0.0144, two-way ANOVA). (B) Odor performance under the spontaneous habituation/dishabituation task. Both animal groups habituated to isoamyl acetate (n = 12 mice per group, $F_{(3, 66)}$ = 6.68, P = 0.0349 and 0.0164, two-way ANOVA). However, PV-*Erbb4*$^{+/+}$ mice ($F_{(1, 22)}$ = 8.93, P = 0.0025), but not PV-*Erbb4*$^{-/-}$ mice (P = 0.8451, two-way ANOVA), dishabituated to limonene. (C) Odor performance under the reversed habituation/dishabituation task. Both animal groups habituated to limonene ($F_{(3, 54)}$ = 16.33, P < 0.0001 and 0.0003, two-way ANOVA). However, PV-*Erbb4*$^{+/+}$ mice ($F_{(1, 18)}$ = 5.22, P = 0.0023), but not PV-*Erbb4*$^{-/-}$ mice (P = 0.7890, two-way ANOVA), dishabituated to isoamyl acetate. (D) PV-*Erbb4*$^{+/+}$ mice were able to detect isoamyl acetate at a concentration of 10^{-5} (n = 10 mice, $F_{(1, 18)}$ = 0.60, 3.74 and 16.69, P = 0.6069, 0.0498, and 0.0096 for 10^{-6}, 10^{-5}, and 10^{-4}, two-way ANOVA). PV-*Erbb4*$^{-/-}$ mice only detected isoamyl acetate at a concentration of 10^{-4} (n = 10 mice, P =

0.5764, 0.5353, and 0.0100 for 10^{-6}, 10^{-5}, and 10^{-4}, two-way ANOVA). (E) PV-*Erbb4*$^{+/+}$ mice could detect limonen at a concentration of 10^{-5} (n = 10 mice, $F_{(1, 18)}$ = 1.75, 7.95 and 32.51, P = 0.4540, 0.0106, and 0.0064 for 10^{-6}, 10^{-5}, and 10^{-4}, two-way ANOVA). PV-*Erbb4*$^{-/-}$ mice only detected limonene at a concentration of 10^{-4} (n = 10 mice, P = 0.2842, 0.2709, and < 0.0001 for 10^{-6}, 10^{-5}, and 10^{-4}, two-way ANOVA). (F) The latency for mice to locate the buried and visible food pellets did not differ between the groups (n = 14 and 11 mice, for the buried food pellet, $F_{(1, 23)}$ = 0.21, P = 0.9786, 0.9279, and 0.8873; for the visible food pellet, P > 0.9999, two-way ANOVA). Data are presented as means ± s.e.m. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001, n.s. = not significant.

Figure 4. Odor-evoked responses in M/TCs are decreased in PV-*Erbb4*$^{-/-}$ mice. (A) Schematic of *in vivo* odor-evoked electrophysiological recordings in awake, head-fixed mice with ErbB4 knocked out in PV interneurons. (B) Representative raw traces of spike activity before (spontaneous), during (odor-evoked), and after 2-s odor stimulation in PV-*Erbb4*$^{+/+}$ and PV-*Erbb4*$^{-/-}$ mice. (C) Examples of raster plots (top) and peristimulus time histograms (PSTHs) of the firing rate (bottom) for odor-evoked excitatory (left) and inhibitory (right) responses in PV-*Erbb4*$^{+/+}$ and PV-*Erbb4*$^{-/-}$ mice. PSTHs were smoothed with a Gaussian filter with a standard deviation of 1500 ms. (D) Heat maps of the mean firing rate (MFR) across all unit–odor pairs in PV-*Erbb4*$^{+/+}$ mice (n = 240 unit–odor pairs from 4 mice) and PV-*Erbb4*$^{-/-}$ mice (n = 232 unit–odor pairs from 4 mice). (E) Quantitative analysis of the spontaneous firing rate ($t_{(470)}$ = 0.2665, P = 0.7899), odor-evoked MFR ($t_{(470)}$ = 3.304, P = 0.0010), absolute

value of odor-evoked changes ($t_{(470)}$ = 5.046, P < 0.0001), and normalized signal-to-noise ratio (SNR) ($t_{(470)}$ = 5.152, P < 0.0001, unpaired t test) across all unit–odor pairs. (F) Odor-evoked excitatory changes in MFR (ΔMFR) for M/TCs recorded from PV-*Erbb4*$^{+/+}$ mice (n = 66 unit–odor pairs from 4 mice) and PV-*Erbb4*$^{-/-}$ mice (n = 16 unit–odor pairs from 4 mice). (G) Quantitative analysis of the spontaneous firing rate ($t_{(80)}$ = 7.666, P < 0.0001), odor-evoked MFR ($t_{(80)}$ = 1.426, P = 0.1578), odor-evoked ΔMFR ($t_{(80)}$ = 3.099, P = 0.0027), SNR ($t_{(80)}$ = 5.909, P < 0.0001), and normalized SNR ($t_{(80)}$ = 5.909, P < 0.0001, unpaired t test) across excitatory unit–odor pairs. (H) Odor-evoked inhibitory ΔMFR for M/TCs recorded from PV-*Erbb4*$^{+/+}$ mice (n = 81 unit–odor pairs from 4 mice) and PV-*Erbb4*$^{-/-}$ mice (n = 148 unit–odor pairs from 4 mice). (I) Quantitative analysis of spontaneous firing rate ($t_{(227)}$ = 7.521, P < 0.0001), odor-evoked MFR ($t_{(227)}$ = 7.228, P < 0.0001), odor-evoked ΔMFR ($t_{(227)}$ = 2.724, P = 0.0070), SNR ($t_{(227)}$ = 6.905, P < 0.0001), and normalized SNR ($t_{(227)}$ = 6.905, P < 0.0001, unpaired t test) across inhibitory unit–odor pairs. (J) ΔMFR for units with no response to odor in PV-*Erbb4*$^{+/+}$ mice (n = 93 unit–odor pairs from 4 mice) and PV-*Erbb4*$^{-/-}$ mice (n = 68 unit–odor pairs from 4 mice). (K) Quantitative analysis of spontaneous firing rate ($t_{(159)}$ = 3.129, P = 0.0021), odor-evoked MFR ($t_{(159)}$ = 3.378, P = 0.0009), absolute value of ΔMFR ($t_{(159)}$ = 1.627, P = 0.1057), and normalized SNR ($t_{(159)}$ = 1.713, P = 0.0886, unpaired t test) across “no response” unit–odor pairs. (L) Distribution of excitatory, inhibitory, and “no response” units in PV-*Erbb4*$^{+/+}$ and PV-*Erbb4*$^{-/-}$ mice ($\chi^2_{(2)}$ =53.85, P < 0.0001, Chi-Square tests). ** P < 0.01, *** P < 0.001, **** P < 0.0001, n.s. = not significant.

Figure 5. The ongoing LFP in the OB is increased in PV-*Erbb4*$^{-/-}$ mice. (A) Examples of ongoing LFP signals recorded in the OB from PV-*Erbb4*$^{+/+}$ and PV-*Erbb4*$^{-/-}$ mice. The five rows show the raw traces and the filtered theta, beta, low-gamma, and high-gamma signals. (B-E) Quantitative analysis of the averaged power spectra in the theta, beta, low-gamma, and high-gamma bands for the two groups. (F-I) Comparisons of power in the theta (n = 5 mice per group, $t_{(8)}$ = 2.80, P = 0.0233, unpaired t test), beta ($t_{(8)}$ = 2.80, P = 0.0232, unpaired t test), low-gamma ($t_{(8)}$ = 3.80, P = 0.0053, unpaired t test), and high-gamma ($t_{(8)}$ = 3.73, P = 0.0058, unpaired t test) oscillations in the OB in the two groups. * P < 0.05, ** P < 0.01.

Figure 6. PV-*Erbb4*$^{-/-}$ mice have impaired information output from the OB. (A and B) Increased frequency of MC spontaneous action potentials (sAPs) and olfactory nerve-evoked APs (eAPs) in PV-*Erbb4*$^{-/-}$ mice, but decreased ratio of eAPs to sAPs (n = 9 from 3 mice per group, sAPs: $t_{(16)}$ = 4.173, P = 0.0007, eAPs: $t_{(16)}$ = 2.24, P = 0.0395; ratio: $t_{(16)}$ = 3.68, P = 0.0020, unpaired t test). (C and D) The eAP-to-sAP ratio was reduced in PV-*Erbb4*$^{-/-}$ mice as the intensity of the stimulus increased (n = 8 from 3 mice per group, $F_{(1, 70)}$ = 32.39, P < 0.0001, two-way ANOVA). (E and F) AP frequency elicited by injection of positive currents was higher in PV-*Erbb4*$^{-/-}$ mice than PV-*Erbb4*$^{+/+}$ mice (n = 9 from 4 and 3 mice per group, $F_{(1, 80)}$ = 78.9, P < 0.0001, two-way ANOVA). Data are presented as means ± s.e.m. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

Figure 7. Decreased GABAergic transmission mediates the OB output

impairments in PV-*Erbb4*$^{-/-}$ mice. (A and B) The increased sAP frequency and decreased ratio of eAPs to sAPs in MCs could not be further enhanced by bicuculline in PV-*Erbb4*$^{-/-}$ mice (n = 7 from 3 PV-*Erbb4*$^{+/+}$ mice, n = 9 from 3 PV-*Erbb4*$^{-/-}$ mice; for sAP, $F_{(1, 14)}$ = 17.08, P = 0.0008 and 0.5531; for ratio of eAPs to sAPs, $F_{(1, 14)}$ = 13.99, P = 0.0007 and 0.9360, two-way ANOVA). (C and D) The frequency but not the amplitude of MC mIPSCs was lower in PV-*Erbb4*$^{-/-}$ mice (n = 9 from 7 PV-*Erbb4*$^{+/+}$ and 6 PV-*Erbb4*$^{-/-}$ mice; for frequency, $t_{(16)}$ = 2.45, P = 0.0263; for amplitude, $t_{(16)}$ = 0.53, P = 0.6038, unpaired t test). (E and F) Neither frequency nor amplitude of MC mEPSCs was different in PV-*Erbb4*$^{-/-}$ mice versus PV-*Erbb4*$^{+/+}$ mice (n = 10 from 5 PV-*Erbb4*$^{+/+}$ mice, n = 9 from 7 PV-*Erbb4*$^{-/-}$ mice; for frequency, $t_{(17)}$ = 0.14, P = 0.8899; for amplitude, $t_{(17)}$ = 0.89, P = 0.3863, unpaired t test). Data are presented as means ± s.e.m. * P < 0.05, *** P < 0.001, **** P < 0.0001, n.s. = not significant.

Figure 8. Reduced recurrent inhibition and EPL lateral inhibition of MCs in PV-*Erbb4*$^{-/-}$ mice. (A and B) Recurrent inhibition did not increase alongside MC hyperactivity in PV-*Erbb4*$^{-/-}$ mice (n = 9 from 5 and 6 mice; for peak amplitude, $t_{(16)}$ = 1.57, P = 0.1351; for decay time constant, $t_{(16)}$ = 0.28, P = 0.7821, unpaired t test). (C and D) Recurrent IPSPs elicited by the same number of MC APs were smaller in PV-*Erbb4*$^{-/-}$ mice. In PV-*Erbb4*$^{-/-}$ mice, an 80 pA current induced ten APs, whereas in PV-*Erbb4*$^{+/+}$ mice, a 100 pA current was needed to induce ten APs. Both the peak amplitude (n = 10 from 6 and 5 mice, $t_{(18)}$ = 2.46, P = 0.0242, unpaired t test) and the decay time constant ($t_{(18)}$ = 2.56, P = 0.0198, unpaired t test) of IPSPs evoked by the

same number of APs were reduced in PV-*Erbb4*$^{-/-}$ mice. (E) Schematic of the EPL lateral inhibition experimental configuration. Whole-cell recording of an MC (right side of the schematic) upon stimulus of an adjacent glomerulus (left side of the schematic). A cut was made through the GL and GCL between the sites of conditioning stimulation and target glomerus to isolate EPL lateral inhibition. (F and G) EPL lateral inhibition was observed in the OB of PV-*Erbb4*$^{+/+}$ mice but not PV-*Erbb4*$^{-/-}$ mice (n = 10 from 7 PV-*Erbb4*$^{+/+}$ mice, $F_{(1, 18)}$ = 26.48, P < 0.0001; n = 10 from 6 PV-*Erbb4*$^{-/-}$ mice, P = 0.3426, two-way ANOVA). (H-J) Cutting through the EPL as well abolished the EPL lateral inhibition in PV-*Erbb4*$^{+/+}$ mice. (H) Schematic of the experimental configuration. (I and J) Representative and quantitative analysis of spike frequency in PV-*Erbb4*$^{+/+}$ mice (n = 9 from 5 mice, $t_{(8)}$ = 0.80, P = 0.4468, paired t test). EPL, external plexiform layer; GL, glomerular layer; GCL, granule cell layer; MCL, mitral cell layer; ONL, olfactory nerve layer; PVN, PV interneuron. Data are presented as means ± s.e.m. * P < 0.05, **** P < 0.0001, n.s. = not significant.

Figure 9. ErbB4 in PV interneurons of the OB is critical for odor discrimination and sensitivity. (A) Schema indicating virus-injection sites. To specifically delete ErbB4 protein in the PV interneurons of the OB, AAV-PV-Cre-GFP was injected into the bilateral OB of neonatal *loxP*-flanked ErbB4 mice. (B) Reduced ErbB4 expression in AAV-PV-Cre-GFP mouse OB (n = 4 mice per group, $t_{(3)}$ = 3.93, P = 0.0293, paired t test). Relative levels were normalized to their respective control groups. (C) The accuracy in discriminating simple odor pairs was similar for the two groups (n = 6 and 7 mice, $F_{(1, 11)}$ = 0.06, P = 0.8147). The accuracy in discriminating difficult odor pairs

was significantly lower in AAV-PV-Cre-GFP mice ($F_{(1, 11)}$ = 5.74, P = 0.0355, two-way ANOVA). (D) Both animal groups habituated to isoamyl acetate (n = 10 mice per group, $F_{(3, 54)}$ = 20.94, P < 0.0001 and = 0.0010, two-way ANOVA). However, the AAV-PV-GFP ($F_{(1, 18)}$ = 3.95, P = 0.0045), but not the AAV-PV-Cre-GFP mice (P = 0.7107, two-way ANOVA), dishabituated to limonene. (E) Both animal groups habituated to carvone+ (n = 10 mice per group, $F_{(3, 54)}$ = 9.92, P = 0.0019 and 0.0035, two-way ANOVA). However, AAV-PV-GFP ($F_{(1, 18)}$ = 6.72, P = 0.0025), but not AAV-PV-Cre-GFP mice (P = 0.9845, two-way ANOVA), dishabituated to carvone-. (F) AAV-PV-GFP mice were able to detect isoamyl acetate at a concentration of 10^{-5} (n = 10 mice, $F_{(1, 18)}$ = 0.04, 2.63 and 12.42, P = 0.8309, 0.0258, and 0.0164 for 10^{-6}, 10^{-5}, and 10^{-4}, two-way ANOVA), whereas AAV-PV-Cre-GFP mice only detected isoamyl acetate at a concentration of 10^{-4} (n = 10 mice, P = 0.9484, 0.8930, and 0.0312, two-way ANOVA). (G) AAV-PV-GFP mice detected limonene at 10^{-5} (n = 10 mice, $F_{(1, 18)}$ = 0.28, 3.81 and 11.79, P = 0.7894, 0.0154, and 0.0131 for 10^{-6}, 10^{-5}, and 10^{-4}, two-way ANOVA) but AAV-PV-Cre-GFP mice only detected limonene at a higher concentration of 10^{-4} (n = 10 mice, P = 0.6408, 0.9349, and 0.0496 for 10^{-6}, 10^{-5}, and 10^{-4}, two-way ANOVA). Data are presented as means ± s.e.m. * P < 0.05, ** P < 0.01, **** P < 0.0001, n.s. = not significant. EPL, external plexiform layer; GCL, granule cell layer; GL, glomerular layer; IPL, internal plexiform layer; MCL, mitral cell layer.